Toll-like Receptor 2 Polymorphisms Impose Considerable Impacts On Acute Myelocytic Leukemi Occurrence

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Abstract

Background: This research aimed to explore the genetic association of Toll-like receptor 2 (*TLR2*) gene polymorphisms with acute myelocytic leukemia (AML) susceptibility in Chinese population.

Methods: Firstly, the genotypes of *TLR2* polymorphism were detected in 148 AML patients and 126 healthy controls by polymerase chain reaction (PCR). The genotype distribution of every polymorphism in the control group was detected whether conformed to Hardy-Weinberg equilibrium (HWE). The genotype frequency difference of *TLR2* polymorphism between the case and control groups was compared by chi-square test and odds ratio (OR) with 95% confidence interval (95%CI) was calculated to express the risk of AML resulted from genetic variant of *TLR2*.

Results: For *TLR2* rs3804099 polymorphism, the heterozygous genotype TC was detected the significantly lower frequency in AML patients than that in the controls ($P=0.031$), so was C allele ($P=0.021$), which showed that rs3804099 was associated with the decreased the risk of AML (OR=0.580, 95%CI=0.354-0.953; OR=0.641, 95%CI=0.438-0.938). Similarly, GG genotype and G allele of rs1898830 also showed the obviously association with the risk reduction of AML (GG vs. AA: OR=0.430, 95%CI=0.200-0.924, G vs. A: OR=0.644, 95%CI=0.451-0.919). However, no significant association was found in rs7656411 with AML.

Conclusions: *TLR2* rs3804099 and rs1898830 polymorphisms may be the protective factors for AML, but not rs7656411.

Background

Acute myelocytic leukemia (AML) is a kind of progressive malignant tumor in hematopoietic system with low cure rate and high recurrence rate [1, 2]. The pathological features of AML are rapidly increasing abnormal leucocytes aggregate in the bone marrow to disturb the production of normal cells such as erythrocytes and platelets [3]. In general, it easily attacks the adults, but 15% of childhood leukemia cases are explained by AML, too [4]. If not timely treatment, AML is fatal in a few months, which brings about huge economic burden and life pressures. The pathogenesis of AML is multifactorial result consisted of environmental and genetic factors [5, 6]. The known environmental factors include smoking, obesity, X radiation and chemical carcinogens [7, 8], however, only a small part of people suffer from AML which expose to risk environmental factors. Therefore, genetic factors play a key role in the onset of AML. Nowadays, identified genes associated with AML are few not to explain the etiology.

Toll-like receptors (TLRs) is a set of conserved pattern recognition receptors which act on innate immune system against pathogenic microorganisms [9]. They are detected the expression in immune cells, including neutrophils, monocytes, dendritic cells, macrophages, T cells, B cells and NK cells [10]. TLRs can influence the secretion of cytokines and chemokines to participate in the immune activity [11, 12]. What's more, the up-regulated expression of TLRs is also detected in several cancers and tumor cell lines [13, 14]. *TLR2* is an important member of TLRs family with the broadest expression in human cells and

recognizes the most types of pathogenic microorganisms. The activation of TLR2 signaling has been found to induce the activation of mitogen-activated protein kinase (MAPK) and NF- κ B so as to prolong the survival time of tumor cells [15]. So far, the researches about the association of TLR2 with AML are few.

TLR2 coding gene *TLR2* is located on chromosome 4q31.3 [16] and had been identified a number of single nucleotide polymorphisms (SNPs). In the present study, we selected three common SNPs in the exon, intron and 3'UTR of *TLR2* to investigate the role in AML occurrence risk in a Chinese Han population.

Methods

Cases and controls

The current study included 148 AML patients and 126 healthy controls as the case and control groups, respectively. In the case group, patients with AML were inpatients from haemal internal medicine of PanYu Central Hospital, including 83 males and 65 females. They were diagnosed based on morphological and phenotypic data according to the criteria of World Health Organization (WHO) [17]. Their age range was from 17 to 69 years old. At the same time, the healthy controls were selected experienced the physical examination in the same hospital with the cases and consisted of 64 males and 62 females with the age of 23-76. The healthy controls were frequency-matched with AML patients in age and sex. All subjects were all Chinese Han population and they were no blood relationship. This study design was reviewed and supported by the Research Ethics Committee of PanYu Central Hospital, meanwhile, the objective and program were informed all subjects before collecting blood samples. Moreover, written consents were signed by every subject.

DNA extraction

2ml peripheral venous blood were collected from every subject with empty stomach on the early morning and placed in the blood collection tube with EDTA2Na, -80°C. Whole blood genomic DNA was extracted using TIANamp Genomic DNA Kit according to the manufacturer's instruction and stored at -20°C for standby application.

The genotyping of *TLR2* polymorphisms

The genotyping of *TLR2* rs3804099, rs1898830 and rs7656411 polymorphisms was conducted by polymerase chain reaction (PCR) with direct sequencing. First of all, PCR primers were designed by Primer Premier 5.0 software and synthesized in Shanghai Sangon Biotech Co., Ltd. The relative information of primer sequences was showed in **Table 1**. PCR system was a volume of 25.0 μ l mixture, including 12.5 μ l PCR Mix, 2.0 μ l DNA template, each 1.0 μ l of forward and reverse primers and finally added ddH₂O to 25.0 μ l. PCR procedure was conducted according to the following steps: pre-degeneration at 95°C for 3min, 33 cycles of degeneration at 95°C for 45s, annealing at 56°C (rs1898830) and 60°C (rs3804099

and rs7656411) for 30s, extension at 72°C for 30s, and final extension at 72°C for 7min. The quality of PCR products was tested by agarose gel electrophoresis.

The eligible PCR products were sent to Sangon Biotech (Sangon, Shanghai) for sequencing so as to determine the genotype of every *TLR2* polymorphism in all subjects.

Statistical analysis

In this study, the genotype frequency was obtained using direct counting and the genotype distribution of every polymorphism in the control group was detected by χ^2 test whether was consistent with Hardy-Weinberg equilibrium (HWE). The genotype, allele frequency difference of *TLR2* polymorphisms between AML patients and the healthy controls were calculated by chi-square test. Odds ratio (OR) and 95% confidence interval (95%CI) was used to represent the relative risk of AML caused by *TLR2* variants. All data were managed by PASW Statistics 18.0 software and showed in the form of $\pm$ s and %.

Table 1
PCR prime sequences of *TLR2* polymorphisms

Polymorphism	Position	Primer sequence		Annealing temperature
rs3804099	Exon3	For.	5'ACTTACCTTCCTTGAGGAACTTG3'	60°C
		Rev.	5'AACTTGTAACATCTACAAAAATCTCCA3'	
rs1898830	Intron1	For.	5'AAATGAATGAGCAAGCAA3'	56°C
		Rev.	5'TGGCCTCCTGCTTATGT3'	
rs7656411	3'UTR	For.	5'-CTTCCTCAGCCTCTAACTACCTT-3'	60°C
		Rev.	5'AAGTTACATAGAATCAGCAAATAGTG3'	
Note: 3'UTR: 3' untranslated region				

Results

The clinical features of subjects

We displayed the clinical features of subjects in the case and control groups in Table 2. The mean age of AML patients and the healthy controls were 50.8 ± 13.4 and 51.6 ± 12.1 , respectively and they were not significantly different ($P=0.803$). The gender distribution of the case and control groups were no obvious difference ($P=0.382$). Total leukocytic count was $8.4 \pm 6.8/\text{mm}^3$ in AML patients and the number was $10.2 \pm 2.6/\text{mm}^3$ in the controls ($P=0.214$). However, there was significant difference in BMI between the case and control groups (24.9 ± 3.4 and 23.8 ± 2.6 , $P=0.000$). 37.84% of AML patients were smokers and the percentage was 29.37% in controls, which suggested smoking was not a influence factor of AML in

this population ($P = 0.140$). Differently, exposed to chemical carcinogens was a risk factor for AML occurrence ($P = 0.024$).

Table 2
The characteristics of the cases and controls

Index	AML patients (n = 148)	The controls (n = 126)	P
Age (years)	50.8 ± 13.4	51.6 ± 12.1	0.803
Gender (male/female)	83/65	64/62	0.382
BMI (kg/m ²)	24.9 ± 3.4	23.8 ± 2.6	0.000
Total leukocytic count/mm ³	8.4 ± 6.8	10.2 ± 3.1	0.214
Smoking	56/37.84	37/29.37	0.140
Exposed to chemical carcinogens (%)	15/10.14	4/3.17	0.024
Note: BMI: body mass index			

HWE test

The status of HWE was checked based on the genotype distribution of polymorphism in the control group, the results were showed in **Table 3**. The genotype distribution of *TLR2* rs3804099, rs1898830 and rs7656411 in the control group were all consistent with HWE requirement ($P=0.161$, 0.466 and 0.245 respectively), indicating our study population was a representative group.

The association analysis of *TLR2* polymorphisms with AML risk

The genotype and allele frequencies of *TLR2* polymorphisms were calculated and displayed in **Table3**. We can see that the genotype and allele distributions of rs3804099 and rs1898830 between the case and control groups were very different, but not rs7656411.

Firstly, the heterozygous genotype TC of rs3804099 was significantly lower frequency in AML patients than that in the controls ($P=0.031$), compared with the common genotype TT and we also obtained the similar results in allele ($P=0.021$). So the carriage of rs3804099 TC genotype and C allele significantly decreased the occurrence risk of AML (TC vs. TT: OR=0.580, 95%CI=0.354-0.953; C vs. T: OR=0.641, 95%CI=0.438-0.938). For rs1898830, both of GG genotype and G allele frequencies in the case group were obviously lower than in the control group ($P=0.028$, 0.015). It was also a protective factors to prevent from AML (GG vs. AA: OR=0.430, 95%CI=0.200-0.924; G vs. A: OR=0.644, 95%CI=0.451-0.919). However, rs7656411 was not detected the significant association with AML risk in genotype or allele ($P>0.05$).

Table 3
The genotype distributions of *TLR2* polymorphisms in the case and control groups

Locus	Genotype/%		OR (95%CI)	P	P _{HWE}
	Case, n = 148	Control, n = 126			
rs3804099					0.161
	TT	87/58.78	56/44.45	1.000 (Ref.)	-
	TC	55/37.16	61/48.41	0.580 (0.354–0.953)	0.031
	CC	6/4.06	9/7.14	0.429 (0.145–1.271)	0.119
	T	229/77.36	173/68.65	1.000 (Ref.)	-
	C	67/22.64	79/31.35	0.641 (0.438–0.938)	0.021
rs1898830					0.466
	AA	76/51.35	49/38.89	1.000 (Ref.)	-
	AG	58/39.19	56/44.44	0.668 (0.400-1.116)	0.123
	GG	14/9.46	21/16.67	0.430 (0.200-0.924)	0.028
	A	210/70.95	154/61.11	1.000 (Ref.)	-
	G	86/29.05	98/38.89	0.644 (0.451–0.919)	0.015
rs7656411					0.245
	GG	54/36.49	34/26.99	1.000 (Ref.)	-
	GT	73/49.32	69/54.76	0.666 (0.388–1.144)	0.140
	TT	21/14.19	23/18.25	0.575 (0.277–1.194)	0.136
	G	181/61.15	137/54.37	1.000 (Ref.)	-
	T	115/38.85	115/45.63	0.757 (0.538–1.064)	0.109

Discussion

The occurrence of AML is caused by karyotype mutation of pluripotent stem cells and lightly differentiated precursor cells [18]. Abnormal chromosome karyotype, especially chromosome translocation is typical cytogenetic characteristic of AML, or else, apparent molecular abnormalities exist in the pathogenesis of AML. The onset of AML is in all ages and it is most common in the elderly, with poor prognosis. Exact explaining the pathology of AML is a urgent issue. AML is a complex multifactorial disease described by precious studies. On the one hand, a series of environmental factors have identified, mainly referring to radiation [19], chemical factor and infection. On the other hand, genetic factors play the key role in the occurrence of AML. So far, multiple gene variants are found to be associated with AML,

including *FLT3-ITD*, *IDH1*, *IDH2*, *DNMT3A* *NPM1* and so on. However, these researches are far away from revealing the etiology of AML.

TLR is one of innate immune receptors and play an important role in activation and regulation of innate immunity, and the induction of adaptive immunity. Meanwhile, it also has an influence on cardiovascular disease, autoimmunity development and tumors [20, 21]. They involve in maintaining the effective immune response in the acute infection and injury. TLR signaling is necessary for normal immune reaction, however, studies have been reported that the abnormality of TLR signaling is related to ineffective hematopoiesis and hematopoietic malignancy [22, 23]. TLR family includes 10 members, of which TLR2 is the broadest one in expression and it also identifies the most pathogenic microorganisms. In previous report, the high expression of TLR2 is found in AML patients and AML patients with high expression of TLR2 have the shorter survival time than that patients with low expression [11]. So, TLR2 may involve in the etiology of AML.

Its encoding *TLR2* is consisted of 5 exons and introns and has been identified a number of SNPs. *TLR2* polymorphisms can influence host innate immune response and immune defence through regulating transcription activity or changing protein structure. Multiple SNPs in *TLR2* are studied the association with some diseases. Rs3804099 is a synonymous mutation in exon3 of TLR2 with the substitution of T/C and it has been found to be associated with several diseases, such as inflammatory disease, cardiovascular disease, immune disease and cancer [24]. Rs1898830 is a mutation of A/G in intron1 region of *TLR2* and the carriage of AA induces the expression of target genes in IKK- β and NF- κ B pathway and promotes the secretion of pro-inflammatory factor, including interleukin and chemokine, such as *TNF*, *IL-6*, *CCL3* and *CCL2* [25, 26]. It also participates in many diseases pathogenesis, certainly including cancer [27]. For rs7656411 is a SNP in 3' untranslated region (3'UTR) of *TLR2* with a base mutation of G/T and it may affect the expression of *TLR2* mRNA.

In the present study, we explored the genetic association of these three common SNPs in *TLR2* with AML susceptibility based on a Chinese Han population. In this study population, smoking was not a risk factor of AML, but obesity and exposed to chemical carcinogens were significantly correlated to the occurrence of AML. Rs3804099 was revealed to be related to the development of AML and the carriage of TC genotype and C allele could significantly decreased the susceptibility of people to AML. Similar results were showed in rs1898830, that is people carrying GG genotype and G allele of rs1898830 had the lower risk suffering from AML, compared with AA genotype and A allele respectively. However, rs7656411 was not checked the obvious association with AML in our study group. This is the first time to investigate the role of *TLR2* multiple SNPs in the risk of AML in Chinese population.

Conclusions

In conclusion, *TLR2* may play an important role in AML pathogenesis and the genetic variants may cause the expression change of *TLR2* to contribute the risk to disease, including AML. We have identified rs3804099 and rs1898830 in *TLR2* associated with AML occurrence based in the current study. But some

limitations influence the final results, too and most of them are small samples, single group and environmental factors. Therefore, more further researches are needed to verify this results with well-design and explore the detailed mechanism in the future.

List Of Abbreviations

Toll-like receptor 2 (*TLR2*)

acute myelocytic leukemia (AML)

polymerase chain reaction (PCR)

Hardy-Weinberg equilibrium (HWE)

odds ratio (OR)

95% confidence interval (95%CI)

Acute myelocytic leukemia (AML)

Toll-like receptors (TLRs)

mitogen-activated protein kinase (MAPK)

single nucleotide polymorphisms (SNPs)

World Health Organization (WHO)

3' untranslated region (3'UTR)

Declarations

Ethics approval and consent to participate

This study was supported by the Ethics Committee of PanYu Central Hospital and also has been carried out in accordance with the World Medical Association Declaration of Helsinki.

The subjects had been informed the objective. Certainly, written consents were signed by every subject in this study.

Consent for publication

We obtaining permission from participants to publish their data.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests The authors declare that they have no competing interests.

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Authors' contributions S.C. design of the work; X.H. the acquisition, analysis, W.Z. interpretation of data; Y.C. the creation of new software used in the work; H.Q. have drafted the work or substantively revised it. All authors read and approved the final manuscript.

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